



## Performance assessment of SARS-CoV-2 IgM & IgG ELISAs in comparison with plaque reduction neutralization test

Ruta Kulkarni<sup>1</sup>, Shubham Shrivastava<sup>1</sup>, Harshad P. Patil<sup>1</sup>, Pravin Kore<sup>1</sup>, Prajakta Rane<sup>1</sup>, Sonali Palkar<sup>2</sup>, Sanjay Lalwani<sup>†</sup>, Akhilesh Chandra Mishra<sup>†</sup> & Vidya A. Arankalle<sup>1</sup>

*Departments of <sup>1</sup>Communicable Diseases, <sup>†</sup>Interactive Research School for Health Affairs & <sup>2</sup>Pediatrics, Bharati Vidyapeeth Medical College, <sup>1</sup>Bharati Vidyapeeth (Deemed to be University), Pune, Maharashtra, India*

**Background & objectives:** Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) continues to be a devastating pandemic. This study was aimed at performance assessment of SARS-CoV-2 IgM and IgG ELISAs, and investigation of their utility for patient diagnosis and sero-epidemiologic investigations.

**Methods:** Serum/plasma samples from COVID-19 patients or asymptomatic contacts (n=180) and healthy donors (n=90) were tested in parallel using two commercial IgM ELISAs (Erbalisa and Inbios), and four IgG ELISAs (Kavach, Euroimmun, Erbalisa and Inbios) along with an indigenous  $\beta$ -propiolactone inactivated virus-based ELISA (IRSHA-IgG-ELISA). Plaque reduction neutralization test (PRNT) was used as reference test.

**Results:** Among 180 COVID-19 patients, 125 tested positive by PRNT. Inbios-IgM-ELISA showed sensitivity (Se)/specificity (Sp)/positive predictive value (PPV)/negative predictive value (NPV) of 93.6/97.8/98.4/94.4 per cent in relation to PRNT, and performed better than Erbalisa-IgM-ELISA (Se: 48%, Sp: 95.6%, PPV: 95.2%, NPV: 65.2%). During the first week of disease, only 47.4 per cent of the COVID-19 patients tested IgM positive by Inbios-IgM-ELISA, detection improving at two weeks and beyond (~86-100%). Among IgG tests, Inbios-IgG-ELISA ranked first in terms of sensitivity (83.2%), followed by IRSHA (64.8%), Euroimmun (64%), Erbalisa (57.6%) and Kavach (56%) tests. For all IgG tests, sensitivity improved during the third (73.9-95.7%) and fourth week (100%) of illness. The specificity (96.7-100%) and PPV (96.2-100%) of all IgG tests were high; NPV ranged between 71.9 and 87.1 per cent with Inbios-IgG-ELISA scoring highest.

**Interpretation & conclusions:** Our results show that IgM detection by the current, most sensitive ELISAs cannot replace molecular diagnosis, but may aid as a supplement test. The available IgG tests are suitable for serosurveys for the assessment of previous virus exposure.

**Key words** Coronavirus disease 2019 - enzyme-linked immunosorbent assays - IgG - IgM - plaque reduction neutralization test - SARS-CoV-2

Coronavirus disease 2019 (COVID-19) caused by infection with severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) was declared as a

pandemic by the World Health Organization on March 11, 2020<sup>1</sup>. Timely and accurate diagnosis is the mainstay of COVID-19 management and control.

Currently, COVID-19 diagnosis is carried out by testing respiratory tract samples for viral RNA using reverse transcription-polymerase chain reaction (RT-PCR)<sup>2</sup>. Although highly sensitive, this method has limitations due to dependence on sampling technique, sample type/quality and virus genetic variability<sup>3</sup>. Further, the performance of RT-PCR is affected by the timing of sample collection relative to the day of illness, as viral RNA is detectable for a limited period post-disease onset<sup>4-6</sup>. Thus, there is a need for sensitive and specific antibody detection tests to supplement molecular diagnosis, particularly if the patients seek medical advice late, when the RNA positivity is bound to be lower. In addition, for seroepidemiologic studies and vaccine immunogenicity testing, IgG tests are crucial.

Since the emergence of the COVID-19 pandemic, extensive efforts have been made for development of antibody detection immunoassays, and several enzyme-linked immunosorbent assays (ELISAs) and lateral flow assays (LFAs) are now commercially available. While the LFAs offer the advantage of rapid results and point-of-care use, their lower sensitivity limits the application of these assays in comparison to ELISAs<sup>7</sup>. The plaque reduction neutralization test (PRNT) remains the gold standard for detection of neutralizing antibodies, however, the test is time-consuming and needs biosafety level 3 (BSL3) facility for handling the live SARS-CoV-2. ELISAs are more suitable for high throughput screening, and allow detection of non-neutralizing antibodies as well.

Performance comparison of SARS-CoV-2 IgM and IgG ELISAs is of special importance for the SARS-CoV-2, for which the antibody dynamics are not yet clearly understood. While initial studies have reported late appearance of antibodies and IgG preceding IgM<sup>6,8-10</sup>, there is a need to revisit this issue by using newer/better tests. The present study was aimed at the assessment of commercially available SARS-CoV-2 IgM and IgG ELISAs, and our indigenously developed IgG ELISA<sup>11</sup> in a clinical setting. In the absence of a reference ELISA recommended by international/national bodies, PRNT was used as the gold standard.

### Material & Methods

This study was conducted at the department of Communicable Diseases, Interactive Research School for Health Affairs (IRSHA), Bharati Vidyapeeth (Deemed to be University), Pune, India, during August

2020. This cross-sectional study was approved by the Institutional Ethics Committee of Bharati Vidyapeeth Medical College (No. IEC/2020/47). A total of 180 serum/plasma samples were obtained from RT-PCR confirmed COVID-19 patients or their asymptomatic contacts admitted at Bharati Hospital and Research Centre, Pune, India, following informed written consent. Blood samples from these patients were collected at different times post-disease onset (0-26 days, average: 10.4 days) and stored at  $-80^{\circ}\text{C}$  till the time of testing. Ninety serum/plasma samples collected from healthy blood donors before the emergence of SARS-CoV-2 (during 2017-2019) were included as negative controls.

*Commercial IgM and IgG ELISAs:* Two commercially available IgM ELISA kits, namely, Erbalisa COVID-19 IgM ELISA (Erbalisa IgM ELISA, Calbiotech, Inc., USA) and SCoV-2 Detect IgM ELISA (Inbios IgM ELISA, Inbios International, Inc., USA), both based on viral spike protein, were evaluated. For IgG detection, four commercially available ELISA kits, namely, Anti-SARS-CoV-2 IgG ELISA (Euroimmun IgG ELISA, Euroimmun Medizinische Labordiagnostika AG, Germany), Erbalisa COVID-19 IgG ELISA (Erbalisa IgG ELISA, Calbiotech, Inc., USA), SCoV-2 Detect IgG ELISA (Inbios IgG ELISA, Inbios International, Inc., USA), anti-SARS CoV-2 IgG Antibody Detection ELISA (Covid Kavach IgG ELISA, Zydus Cadila Healthcare Limited, India) were assessed. COVID Kavach IgG ELISA (hereafter referred to as Kavach IgG ELISA) uses gamma-irradiated inactivated virus, whereas the spike protein is employed by the other three IgG tests.

*Indigenous inactivated whole virus-based IgG ELISA:* SARS-CoV-2 isolation, propagation and inactivation using 0.1 per cent  $\beta$ -propiolactone (Sigma-Aldrich, Inc., Saint Louis, MO, USA) was carried out in the BSL3 facility of IRSHA. The inactivated virus was used as the coating antigen for an indirect IgG ELISA, hereafter referred to as IRSHA IgG ELISA. The ELISA protocol described previously<sup>11</sup> was followed.

*Plaque reduction neutralization test:* SARS-CoV-2 PRNT was performed in IRSHA BSL3 laboratory using Vero CCL81 cells procured from ATCC and maintained in minimum essential medium (MEM; Gibco, Waltham, MA, USA) with 10 per cent foetal bovine serum (FBS; Gibco, Waltham, MA, USA) and antibiotics including penicillin-streptomycin

(100 µg/ml; Thermo Fisher Scientific, Waltham, MA, USA) at 37°C in a humidified incubator with five per cent carbon dioxide. For the assay, cells were seeded at a density of  $1 \times 10^5$  cells/well in a 24-well plate, one day prior to infection. Serum samples diluted 1:5 (v/v) in MEM containing two per cent FBS and antibiotics, were subjected to heat inactivation followed by 4-fold serial dilutions. Each dilution was mixed with equal volume of 20-40 pfu of SARS-CoV-2, followed by incubation at 37°C. After one hour, each virus-serum mixture was added in duplicate wells of the seeded 24-well plate, and incubated for one hour, followed by the addition of overlay medium containing MEM, one per cent carboxymethyl cellulose (Aquacide-II, Merck, Calbiochem-Merck, San Diego, CA, USA), two per cent FBS and antibiotics. At five days post-infection, cells were fixed using 3.7 per cent formaldehyde and stained using one per cent crystal violet (Sigma-Aldrich, Inc., Saint Louis, MO, USA). Plaques were counted and PRNT<sub>50</sub> titre was determined using Karber's formula<sup>12</sup>. Samples with PRNT<sub>50</sub> titre  $\geq 20$  were considered seropositive.

*Statistical analysis:* Sensitivity, specificity, positive and negative predictive values (PPV and NPV) of the IgM and IgG ELISAs were assessed against PRNT as the reference test. Uncertainty was expressed by 95 per cent confidence intervals (95% CI). Proportions were compared using Chi-square test. The analyses were conducted using RStudio version 3.4.1 (RStudio, Inc., Boston, MA, USA).

## Results

*Detection of SARS-CoV-2 antibodies using PRNT:* Among RT-PCR confirmed COVID-19 patients, neutralizing antibody positivity was 69.4 per cent (125/180). The antibody detection rate increased over the course of illness – Week 1: 38/76 (50%), Week 2: 54/63 (85.7%), Week 3: 23/23 (100%), Week 4: 5/5 (100%) (Table I). PRNT did not detect anti-SARS-CoV-2 antibodies in the serum/plasma samples of 90 healthy blood donors obtained prior to the emergence of COVID-19.

*Comparison of ELISAs for the detection of SARS-CoV-2 antibodies among COVID-19 patients:*

**IgM ELISAs:** Among the 125 PRNT-positive samples, 60 (48%) and 117 (93.6%) tested positive, respectively

using the Erbalisa and Inbios IgM ELISAs, with the IgM positivity rising with increase in post-onset day (POD) of disease (Table I). The Inbios ELISA detected IgM in eight PRNT-negative samples, while for the Erbalisa kit this number was higher (n=19). Of these, five IgM-reactive samples were common to both ELISAs.

**IgG ELISAs:** For IgG detection, 81 (64.8%), 70 (56%), 80 (64%), 72 (57.6%) and 104 (83.2%) of the PRNT-positive samples tested positive, respectively using IRSHA, Kavach, Euroimmun, Erbalisa and Inbios IgG ELISAs, with the IgG detection improving steadily over the course of the illness (Table I). Among the 55 PRNT-negative samples, 3-5 were positive for IgG using different ELISAs; the samples identified as IgG-reactive were different in these ELISAs. These IgG ELISA-positive, PRNT-negative samples could represent the presence of non-neutralizing antibodies not detected by PRNT, or ELISA false positivity.

*Comparison of performance characteristics of SARS-CoV-2 IgM and IgG detection ELISAs with PRNT as the reference test:* To assess specificity and PPV of the different ELISAs, 90 blood donor serum/plasma samples obtained prior to the emergence of COVID-19, and tested negative by PRNT were used (Table II). With four and two donor samples testing positive, the specificity of the Erbalisa and Inbios IgM tests was 95.6 and 97.8 per cent, while their PPV was 95.2 and 98.4 per cent respectively. The Kavach, Euroimmun and Inbios IgG ELISAs demonstrated 100 per cent specificity and PPV. The IRSHA and Erbalisa IgG tests respectively identified one and three healthy donor samples as SARS-CoV-2 IgG-positive, and thus, their specificity was 98.9 and 96.7 per cent, while PPV was 98.9 and 96.2 per cent, respectively.

Among the IgM tests, the Inbios ELISA showed significantly higher sensitivity (93.6 vs. 48%,  $P < 0.001$ ) and NPV (94.4 vs. 65.2%,  $P < 0.001$ ) as compared to the Erbalisa test (Table II). The test performance was further analysed in relation to POD of disease. During the first week of illness, the sensitivity of Inbios IgM ELISA was as high as 86.8 per cent and significantly better than that of the Erbalisa IgM ELISA (31.6%,  $P < 0.001$ ). Of the 38 PRNT positives identified during the first week, 33 (86.8%) were identified as IgM positive by Inbios assay; while five samples were PRNT alone and three were IgM alone positive (Table I). The Inbios IgM ELISA continued to show higher sensitivity as compared to the Erbalisa test during the second (94.4

**Table I.** Comparative performance of ELISA for detection of SARS-CoV-2 IgM and IgG antibodies in relation to post-onset day of disease, with plaque reduction neutralization test as the reference test

Name of the ELISA (coating antigen used)	PRNT result	ELISA result	Number of samples					Total (n=180)
			POD 0-7 (n=76)	POD 8-14 (n=63)	POD 15-21 (n=23)	POD 22-28 (n=5)	Asymptomatic (n=13)	
Erbalisa IgM (S2 domain of spike protein)	Positive	Positive	12	28	15	3	2	60
	Negative	Negative	27	4	0	0	5	36
	Positive	Negative	26	26	8	2	3	65
	Negative	Positive	11	5	0	0	3	19
Inbios IgM (spike protein)	Positive	Positive	33	51	23	5	5	117
	Negative	Negative	35	6	0	0	6	47
	Positive	Negative	5	3	0	0	0	8
	Negative	Positive	3	3	0	0	2	8
IRSHA IgG (BPL-inactivated virus)	Positive	Positive	17	35	21	5	3	81
	Negative	Negative	35	9	0	0	6	50
	Positive	Negative	21	19	2	0	2	44
	Negative	Positive	3	0	0	0	2	5
Kavach IgG (gamma-irradiated inactivated virus)	Positive	Positive	14	29	20	5	2	70
	Negative	Negative	35	9	0	0	7	51
	Positive	Negative	24	25	3	0	3	55
	Negative	Positive	3	0	0	0	1	4
Euroimmun IgG (S1 domain of spike protein)	Positive	Positive	15	37	21	5	2	80
	Negative	Negative	36	9	0	0	7	52
	Positive	Negative	23	17	2	0	3	45
	Negative	Positive	2	0	0	0	1	3
Erbalisa IgG (spike protein)	Positive	Positive	16	31	17	5	3	72
	Negative	Negative	36	9	0	0	7	52
	Positive	Negative	22	23	6	0	2	53
	Negative	Positive	2	0	0	0	1	3
Inbios IgG (spike protein)	Positive	Positive	27	46	22	5	4	104
	Negative	Negative	37	8	0	0	7	52
	Positive	Negative	11	8	1	0	1	21
	Negative	Positive	1	1	0	0	1	3

POD, post-onset day of symptoms; PRNT, plaque reduction neutralization test

vs. 51.9%,  $P<0.001$ ) and third week of illness (100 vs. 65.2%,  $P=0.002$ ) (Table II).

For IgG detection, the Inbios ELISA showed the highest sensitivity (83.2%,  $P<0.05$ ), followed by IRSHA (64.8%), Euroimmun (64%), Erbalisa (57.6%) and Kavach (56%) ELISAs (Table II). The NPV of the Inbios IgG ELISA (87.1%) was significantly higher than the other four tests (71.9-76%,  $P<0.05$ ). On analysis in relation to POD, the test sensitivity ranged between 36.8 and 71.1 per cent for the IgG ELISAs during the first week of illness, improved steadily

during the second (53.7-85.2%) and third week (73.9-95.7%). During the fourth week, only five samples were available, and all tested positive with each of the ELISAs evaluated. The Inbios ELISA detected IgG in higher proportion of the PRNT-positive samples during the first (71.1%) and second week of illness (85.2%), as compared to the other four tests, and was thus the most sensitive assay in early disease phase ( $P<0.05$ ).

*Comparison of IgM and IgG markers for the detection of SARS-CoV-2 infection:* On comparison of the IgM (Inbios ELISA) and IgG (Inbios ELISA) markers for

**Table II.** Performance characteristics of SARS-CoV-2 antibody detection ELISAs with plaque reduction neutralization test as the reference test

Name of the ELISA	Sensitivity in relation to post-onset day of disease %, (95% CI)			
	POD 0-7	POD 8-14	POD 15-21	POD 22-28*
Erbalisa IgM	31.6 (16.8-46.4)	51.9 (38.5-65.2)	65.2 (45.8-84.7)	60.0 (17.1-102.9)
Inbios IgM	86.8 (76.1-97.6)	94.4 (88.3-100.6)	100.0	100.0
IRSHA IgG	44.7 (28.9-60.6)	64.8 (52.1-77.6)	91.3 (79.8-102.8)	100.0
Kavach IgG	36.8 (21.5-52.2)	53.7 (40.4-67.0)	87.0 (73.2-100.7)	100.0
Euroimmun IgG	39.5 (23.9-55.0)	68.5 (56.1-80.9)	91.3 (79.8-102.8)	100.0
Erbalisa IgG	42.1 (26.4-57.8)	57.4 (44.2-70.6)	73.9 (56.0-91.9)	100.0
Inbios IgG	71.1 (56.6-85.5)	85.2 (75.7-94.7)	95.7 (87.3-104.0)	100.0

  

Name of the ELISA	Sensitivity in relation to post-onset day of disease %, (95% CI)		Specificity %, (95% CI)	PPV %, (95%CI)	NPV %, (95%CI)
	Asymptomatic	Total			
Erbalisa IgM	40.0 (2.94-82.9)	48.0 (39.2-56.8)	95.6 (91.3-99.8)	95.2 (90.6-99.8)	65.2 (58.4-72.1)
Inbios IgM	100.0	93.6 (89.3-97.9)	97.8 (94.7-100.8)	98.4 (96.3-100.6)	94.4 (90.6-98.2)
IRSHA IgG	60.0 (17.1-102.9)	64.8 (56.4-73.2)	98.9 (96.7-101.1)	98.9 (96.6-101.1)	76.0 (69.8-82.2)
Kavach IgG	40.0 (2.94-82.9)	56.0 (47.3-64.7)	100.0	100.0	71.9 (65.6-78.2)
Euroimmun IgG	40.0 (2.94-82.9)	64.0 (55.6-72.4)	100.0	100.0	75.9 (69.8-82.1)
Erbalisa IgG	60.0 (17.1-102.9)	57.6 (48.9-66.3)	96.7 (93.0-100.4)	96.2 (91.9-100.4)	72.4 (66.1-78.7)
Inbios IgG	80.0 (44.9-115.1)	83.2 (76.6-89.8)	100	100.0	87.1 (82.0-92.3)

\*Only 5 samples were available at the time point. POD, post-onset day of symptoms; PPV, positive predictive values; NPV, negative predictive value; CI, confidence interval

COVID-19 diagnosis among 180 patients, the detection rate of IgM ELISA (125/180, 69.4%) was found to be higher than that of the IgG ELISA (107/180, 59.4%,  $P < 0.05$ ). When both the antibodies were considered, 127/180 (70.6%) were circulating virus-specific antibodies by Inbios ELISA. Among these, 105 were positive for both IgM and IgG, while 20 and two samples were, respectively, positive for IgM alone and IgG alone. Of the 20 IgM-alone positives, 15 were positive for PRNT, suggesting that IgM antibodies may have neutralization potential. Both the IgG-alone positives were PRNT-positive.

### Discussion

The present study reports comparative assessment of two commercial SARS-CoV-2 IgM and four IgG ELISAs along with one indigenous IgG ELISA using PRNT as the reference test. While PRNT detects only neutralizing antibodies, ELISAs detect both neutralizing and non-neutralizing (binding) antibodies.

Diagnostic utility of serologic testing in COVID-19 has been questionable because of the observations of delayed antibody response and IgG appearance

before IgM<sup>6,8-10</sup>. Our data revealed that Inbios IgM ELISA showed very good sensitivity in relation to PRNT. However, only 47.4 per cent (36/76) of the RT-PCR positives identified during the first week of disease were IgM positive, PRNT identifying 50 per cent. Thus, utility of Inbios IgM ELISA as a single diagnostic test would have limited application for diagnosis during the first week of disease, when viral RNA is likely to be detected. Blood collection and IgM/PRNT testing among the COVID-19 patients was done after confirmation of viral RNA positivity (1-4 days later), and hence possibility of RNA negativity at the time of blood collection cannot be ruled out. Despite this apparent lower detection rate in early disease phase, the Inbios IgM ELISA could still be considered for diagnosis in remote places or wherever RNA testing facilities are not available. During the second week of disease, when RNA positivity is bound to be lower, the Inbios IgM ELISA detection improved to about 86 per cent. Therefore, for patients seeking medical advice post-one week of clinical symptoms, IgM ELISA may be the preferred diagnostic test. Importantly, 91.1 per cent agreement was noted between IgM (binding antibodies in ELISA) and neutralizing (PRNT) antibodies. The performance of Erbalisa IgM

ELISA was inferior to the Inbios test. Both the IgM tests demonstrated high specificity (>95%). For the Epitope Diagnostics IgM ELISA, 17.9 per cent positivity has been documented during the first five days, increasing to 52.8 and 79.4 per cent, respectively, at 6-10 and 11-15 days post-disease onset<sup>13</sup>. Thus, to assess diagnostic utility of IgM testing, IgM detection and RNA testing should be done simultaneously.

During the current pandemic, efficient IgG detection is of paramount importance for identification of virus exposure among contacts of symptomatic patients, high risk groups as well as for population-based serosurveys and vaccine evaluations. In the present study, the Inbios IgG ELISA ranked first in terms of sensitivity (83.2%,  $P < 0.05$ ), followed by IRSHA (64.8%), Euroimmun (64%), Erbalisa (57.6%) and Kavach (56%) tests. Importantly, the Inbios ELISA showed >70 per cent sensitivity in the first week of illness, indicating its potential for early IgG detection. The data reveal that if sensitive ELISAs are used, antibodies can be detected early during the disease. For all the ELISAs, sensitivities improved during the third week of illness (73.9-95.7%). In the fourth week, only five samples were available and all were IgG positive. The test sensitivities determined in relation to PRNT confirm earlier reports of superiority of cell-based neutralization tests over IgG ELISAs<sup>14,15</sup>.

On comparison of IgG and IgM markers using Inbios ELISAs, the most sensitive tests identified during the present study, IgM positivity was higher than IgG during the first and second weeks of disease, suggestive of IgM as a useful marker for current/recent SARS-CoV-2 infection. This is in contrast to earlier observations suggesting late appearance of anti-SARS-CoV-2 IgM, and thus limited utility for diagnosis<sup>6,8-10,16,17</sup>. Our data thus suggest that when sensitive and specific tests are employed, antibody dynamics of SARS-CoV-2 appears to be similar to other viral infections, IgM appearing before IgG and serving as a marker of recent infection.

A previous study comparing three commercial IgG ELISAs using concurrent positivity or negativity as the criteria for accuracy demonstrated sensitivity/specificity of 86.4/96.2 per cent (Euroimmun), 100/88.7 per cent (Epitope Diagnostics Inc, USA) and 86.4/100 per cent (Mikrogen Diagnostik, Germany) among COVID-19 patient samples at 11.9 days ( $\pm 5.0$  days) post-onset of symptoms<sup>18</sup>. Using microneutralization test (MNT) as a gold standard, specificity/sensitivity of IgG immunoassays were determined to be 95.1/80.5 per cent (Abbott Architect, Randox Laboratories Ltd.,

UK), 94.9/43.8 per cent (Liaison®, Diasporin, USA) and 86.6/70.7 per cent (Euroimmun)<sup>14</sup>. Thus, when neutralization tests were used as comparator for IgG ELISAs, the sensitivity of Euroimmun ELISA varied from 70.7 to 64 per cent (present study). Other studies have reported 67.1-81.5 per cent IgG detection in COVID-19 patients by Euroimmun ELISA<sup>7,15,19,20</sup>. For the Kavach IgG ELISA, sensitivity/specificity of 92.4/97.9 per cent were reported when the results on samples collected after the second week of disease onset were compared with MNT<sup>21</sup>. A lower sensitivity of 75.7 per cent was documented subsequently<sup>22</sup>. Similar data for other tests evaluated by us are not available. The performance of the evaluated ELISAs was not dependent on the type of antigen used for coating, as evident from the differences in sensitivity among the tests employing the same antigen; IRSHA and Kavach ELISAs used inactivated virus, while Inbios and Erbalisa tests used spike protein. The method of antigen preparation may have affected their performance.

Comparison of the ELISAs with PRNT allowed us to assess the ability of different ELISAs in identifying samples with neutralizing antibodies. Our data revealed low ELISA positivity among the PRNT-negative COVID-19 patient samples. Based on our data, it may be surmised that for comparing both IgM and IgG tests, samples collected during the first and second week post-infection should be used as almost 100 per cent positivity is achieved thereafter by all the tests.

Our study had certain limitations. First, RT-PCR-based diagnosis and blood collection for antibody testing was not done on the same day. The possibility of viral RNA negativity on the day of blood collection cannot be ruled out. Thus, the diagnostic utility of IgM cannot be truly determined. Second, cross-reactivity of the different ELISAs with closely related coronaviruses causing common cold such as HCoV-OC43, HCoV-NL63, HCoV-229E, HCoV-HKU1, could not be investigated due to the unavailability of known serum samples positive for antibodies against these viruses.

In conclusion, IgM detection by the currently available, most sensitive ELISAs cannot replace RT-PCR, but may prove useful as a supplement to molecular diagnosis. The available IgG tests should suffice the current need of assessment of previous exposure. In our study, Inbios IgM and IgG ELISAs provided optimum performance.

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**Conflicts of Interest:** None.

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*For correspondence:* Dr Vidya A. Arankalle, Department of Communicable Diseases, Interactive Research School for Health Affairs, Bharati Vidyapeeth (Deemed to be University), Pune-Satara Road, Katraj-Dhankawadi, Pune 411 043, Maharashtra, India  
e-mail: varankalle@yahoo.com